line 10 (minimum peptide length of amino acids); at page 7, line 15 (peptide has fewer than 100 amino acids); page 5, lines 5-6 (further definition of a portion); page 5, lines 10-11 (further definition of a variant). Support for the amendment to claim 19 can be found, for example, at page 20, lines 21-22 (vaccine is for a cancer in which WT-1 is aberrantly expressed). New claim 39 is dependent from claim 1, but excludes portions and variants. Support for new claim 40 can be found, for example, at page 9, line 10 (peptide has 8 to 12 amino acids). Support for new claim 41 can be found, for example, at page 6, lines 9-11 (peptide capable of being processed by an antigen presenting cell so that a fragment is produced which is able to bind to HLA-A0201). Support for new claim 42 can be found, for example, in claim 1 and at page 9, line 10. Support for new claim 43 can be found, for example, in claim 42, but has been restricted to exclude variants. Support for new claim 44 can be found, for example, at page 8, lines 26-27 (cancer is leukaemia, breast, melanoma or ovarian).

The specification has been amended to capitalize the disclosed trademarks, thereby conforming with M.P.E.P. 608.01(V). No new matter has been added.

The present invention is directed to CTL recognized peptide epitopes. The claimed peptides are processed so that a fragment (at least the minimal sequence recognized by the CTL) is produced which is able to bind to an appropriate MHC molecule and be presented by a cell to elicit a suitable T cell response.

U.S.S.N. 09/625,963
Filed: July 26, 2000
AMENDMENT AND RESPONSE TO OFFICE ACTION

Rejection Under 35 U.S.C. § 112, first paragraph

Claims 1, 4-7, 15 and 19 were rejected under 35 U.S.C. § 112, first paragraph, as not being enabled. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The Examiner has rejected all of the claims because allegedly "the specification does not reasonably provide enablement for the recitation of a cancer *vaccine* for *any* cancer". Please note that only claim 19 (and new claim 44) relate to a cancer vaccine. Claim 19 has been amended to refer to a cancer in which WT-1 is aberrantly expressed. Therefore, claim 19 is no longer directed to *any* cancer.

The Examiner has rejected all of the claims on the grounds that the present application fails to provide guidance for the production of peptides larger than a peptide consisting of SEQ ID NO:1 that can bind to a class I molecule. This rejection is based on Janeway *et al* ("Janeway"). Applicants respectfully submit that there are no grounds provided by Janeway for this rejection. The teachings of Janeway actually show that there is no strict size limitation on the peptides capable of binding to class I molecules. For example, page 121 merely states that "peptides that bind to MHC class I are usually (i.e. not always) 8-10 amino acids long". Further adding that "the peptide is able to extend out of the groove" and that "some length variation may also be accommodated in this way". In view of the foregoing discussion, it is clear that Janeway does not limit MHC binding to peptides of 8-9 amino acids in length. Furthermore, peptides according to claim 1 having a greater number of amino acids than SEQ ID NO:1 would typically be processed by an antigen presenting cell to produce a fragment that binds to an MHC molecule

(please see page 6, lines 9-12; new claim 41; and paragraph bridging pages 122 and 123 of Cellular and Molecular Immunology (2nd edition, Abbas, Lichtman, and Pober, 1994).

Claims 1, 4-6, 15 and 19 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventor had possession of the claimed invention. Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

The motif of SEQ ID NO:1 in a peptide represents the conserved region critical for structural and specific immunoprotective functional features. For this very reason, one of ordinary skill in the art will appreciate that the presence of this motif in a peptide is all that is required to teach how to make a peptide with these features. It is well within the ability of a person of ordinary skill in the art to make peptides comprising a portion or variant of SEQ ID NO:1, as defined by claim 1, and to test whether the peptides retain the ability to bind HLA-A2 using entirely routine methods and techniques available at the time of filing the present application (please see, for example, page 40 of the specification).

The motif represented by SEQ ID NO:1 is bound by the HLA molecule and presented on the surface of the cell. If the peptide is larger than the 9 amino acid sequence disclosed as SEQ ID NO:1, the residues that lie outside of the contiguous 9 amino acid sequence may be fragmented by the suitable antigen presenting cell (see page 6, lines 9-12). The flanking residues (often a carrier protein or peptide – see page 6, lines 19-20) do not substantially affect the ability of the peptide, representing the conserved region critical for the structural and specific

immunoprotective functional features, to bind to the MHC molecule or to present the peptide to the CTL (page 9, lines 17-22). Submitted with this amendment and response, are two U.S. patents (U.S. Patent No. 6,348,584 B1 and U.S. Patent No. 6,335,424 B1) which illustrate the use of the terminology "peptide comprising the amino acid ....." in the issued claims, thereby providing evidence that such terminology is entirely conventional in the art.

Applicants submit that the claims as amended define the terms "portion" and "variant", wherein "portion" refers to the peptide having at least 6 consecutive amino acids from SEQ ID NO:1, and wherein "variant" refers to a peptide having the side chains of one or two of the amino acids of SEQ ID NO:1 altered.

Applicants respectfully submit that the Examiner's rejection of claim 6 is without basis. The Applicants teach at page 4, lines 10-25, the use of non-peptide bonds as alternatives to amino acid residues joined by peptide linkages.

Rejection Under 35 U.S.C. § 102

Claims 1 and 19 were rejected under 35 U.S.C. § 102(b) as being anticipated by any one of Gene 175: 167-172, 1996 by Semba *et al* ("Semba"); Cancer Res. 52:6407-6412, 1992 by Sharma *et al.* ("Sharma"); Mol. Cell. Biol. 11:1707-1712, 1991 by Buckler *et al.* ("Buckler"); or Eur. J. Biochem. 220:395-402, 1994 by Bluyssen *et al.* ("Bluyssen"). Applicants respectfully traverse this rejection to the extent that it is applied to the claims as amended.

Claim 1 has been amended to restrict the length of the peptide to at least 8 but fewer than 100 amino acids. None of the cited references disclose peptides according to claim 1 as amended. Therefore claim 1 is novel. With the exception of claims 7, 42, and 43, all other

claims depend from claim 1. Claim 7 is limited to a peptide consisting of the exact sequence of SEQ ID NO:1, whereas claims 42 and 43 are limited to peptides having from 8 to 12 amino acids. There is no disclosure of a peptide that falls within the scope of any of claims 7, 42, and 43 in any of the cited documents. Therefore, all claims of the application are novel.

U.S.S.N. 09/625,963
Filed: July 26, 2000
AMENDMENT AND RESPONSE TO OFFICE ACTION

Allowance of claims 1, 4-7, 15, 19 and 39-44 is respectfully solicited.

Respectfully submitted,

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Aisha Wyatt

Date: February 28, 2002

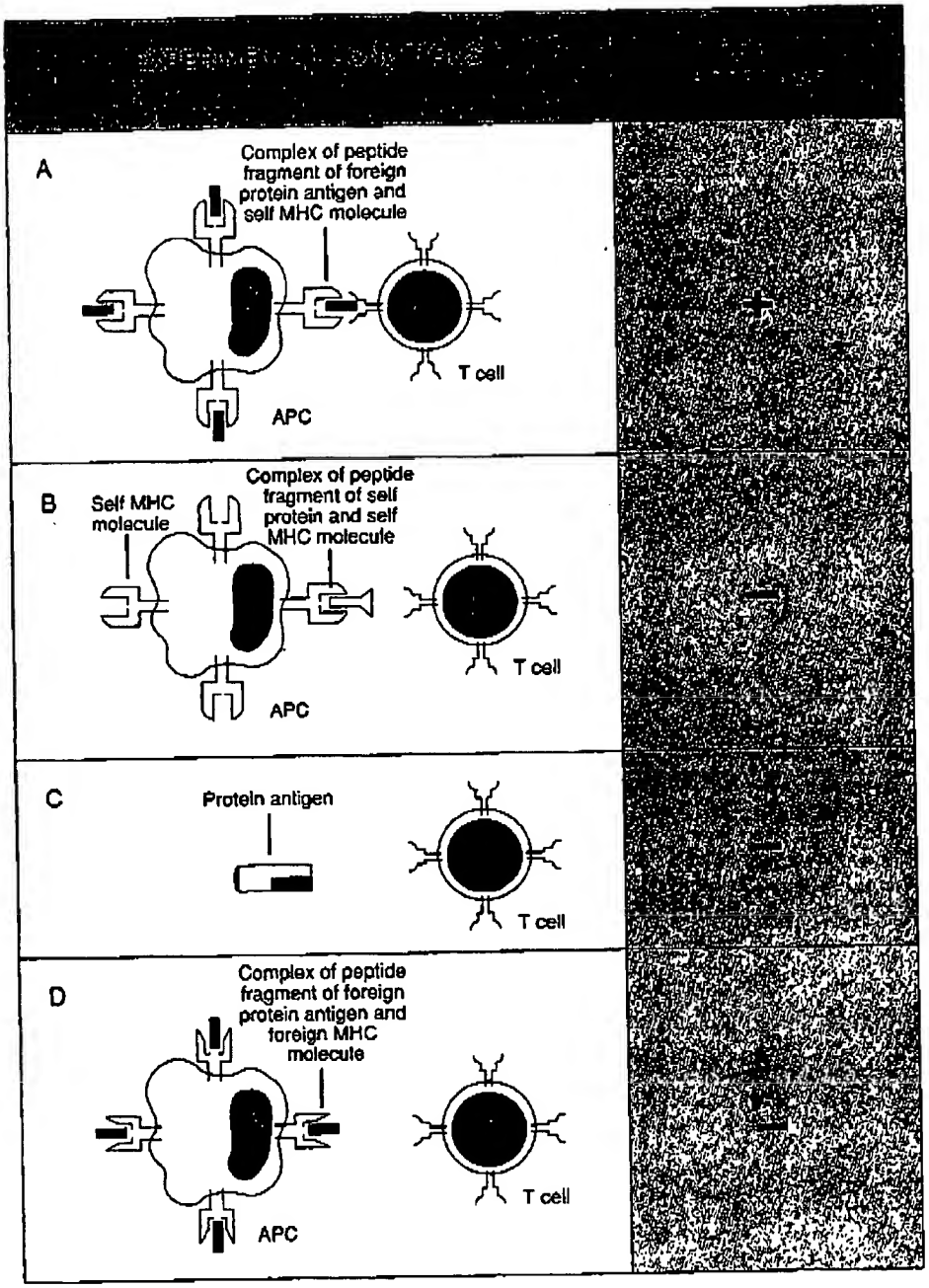

FIGURE 6–4. Specificity of MHC-restricted T cells. *Helper T cells and cytolytic T lymphocytes (CTLs) recognize complexes of self MHC molecules and peptide fragments of foreign antigens (A). MHC-restricted T cells do not recognize self MHC with self peptide (B), foreign antigens without MHC molecules (C), or complexes of foreign MHC molecules and peptide fragment of antigen (D). APC, antigen-presenting cell; MHC, major histocompatibility complex. (Note that some MHC molecules in this and other figures are depicted without bound peptides for the sake of clarity. Most MHC molecules actually do have bound self peptides.)*

tion was formally established when techniques for stimulating immune responses *in vitro* were developed. For example, T cells isolated from the blood, spleen, or lymph nodes of individuals immunized with a protein antigen can be restimulated in tissue culture by that antigen. Stimulation may be measured by assaying the production of cytokines by the T cells or by the proliferation of the T cells. When contaminating macrophages and dendritic cells are removed from the cultures, the purified T lymphocytes no longer respond to antigen, and responsiveness can be restored by adding back the macrophages or dendritic cells. Such experimental approaches provide the basis for defining the accessory functions of various cell types in T lymphocyte activation. The importance of accessory cells in immune responses *in vivo* is suggested by the observation that adjuvants often need to be administered in addition to antigen in order to elict an immune response to the antigen. These adjuvants are usually insoluble or undegradable substances that promote nonspecific inflammation, with recruitment of mononuclear phagocytes at the site of immunization.

Access ry cells serve two important functions In the activation of $CD4^+$ T cells. First, accessory cells are APCs, i.e., they convert protein antigens to peptides and they present peptide-MHC complexes in a form that can

be recognized by CD4+ T cells. The conversion of native proteins to MHC-associated peptide fragments by APCs is called **antigen processing.** As early as the 1950s, it was demonstrated that radioactively or fluorescently labeled antigens injected into animals were found in mononuclear phagocytes or follicular dendritic cells and not in lymphocytes. Later studies showed that an antigen that was taken up by macrophages *in vitro* and then injected into mice was up to 1000 times more immunogenic on a molar basis than the same antigen administered by itself, in a cell-free form. The explanation for this finding is that T cells respond only to antigen associated with macrophages or other APCs, and only a small fraction of an injected soluble antigen ends up in this processed, immunogenic cell-associated form.

The second function of accessory cells is to provide stimuli to the T cell, beyond those initiated by peptide-MHC complexes binding to the T cell antigen receptor. These stimuli, referred to as **costimulator activities,** are required for full physiologic activation of the T cells and are provided by membrane-bound or secreted products of accessory cells. In fact, adjuvants may enhance immune responses *in vivo* in part by inducing the expression of costimulator molecules on accessory cells. The antigen-presenting functions of accessory cells are discussed in more detail in this portion of the chapter, and their costimulator functions are discussed in Chapter 7.

Types of Antigen-Presenting Cells

The two requisite properties that allow a cell to function as an APC for class II MHC–restricted helper T lymphocytes are the ability to process endocytosed antigens and the expression of class II MHC gene products. Most mammalian cells appear to be capable of endocytosing and processing protein antigens, so that the critical property that enables a particular cell to function as an APC is the expression of class II MHC molecules (Table 6–4).

The best-defined APCs for helper T lymphocytes include: (1) mononuclear phagocytes, (2) B lymphocytes, (3) dendritic cells, (4) Langerhans cells of the skin, and (5) in humans, endothelial cells (Table 6–5).

Macrophages and other cells of the **mononuclear phagocyte system** actively phagocytose large particles. Therefore, they probably play an important role in presenting antigens derived from infectious organisms such as bacteria and parasites. Macrophages not only serve as APCs for antigens derived from certain microorganisms, but they also are important effector cells for the killing of these microorganisms. Macrophage presentation of microbial antigens to some CD4+ T lymphocytes results in the secretion of the cytokine interferon-γ (IFN-γ) by the T cells. IFN-γ then activates the macrophages to become more effective killers of microorganisms. (The biologic activities of IFN-γ are described in Chapter 12.) This ability of macrophages to both stimulate and respond to T cells provides an amplification mechanism that increases the ability of the specific immune system to deal with infections.

B lymphocytes specific for a protein antigen are very efficient at presenting that antigen to helper T lymphocytes *in vitro* and may serve as APCs *in vivo*, particularly when the concentration of available antigen is low. The reason why antigen-specific B cells are highly efficient APCs is that their membrane Ig molecules can bind the antigen with high affinity and, therefore, at low concentrations. Ig-bound antigen is also efficiently endocytosed and delivered to intracellular sites of processing (discussed below). The antigen-presenting function of B cells is particularly important in helper T cell–dependent antibody production (see Chapter 9).

Dendritic cells of the spleen and lymph nodes are irregularly shaped, nonphagocytic cells making up a small fraction (<1 per cent) of the total cell population of these organs. They are derived from the bone marrow and may be related to the mononuclear phagocytic lineage (see Chapter 2). Dendritic cells are competent at presenting protein antigens to helper T cells, including naive T cells that have not previously been exposed to antigen. It is also believed that dendritic cells are important for inducing T cell responses to foreign (allo-

TABLE 6–4. Requirement for Class II MHC Expression in Antigen Presentation to CD4+ Antigen-Specific T Cells

APCs	Genes Transfected Into APCs	Surface Class II MHC	Surface Class I MHC	Antigen	Response of Cytochrome c–Specific, I-E^k–Restricted T Cell Line (Cytokine Secretion)
3T3 (murine fibroblast)	None	None	K^k, D^k	Cytochrome c	–
3T3 (murine fibroblast)	Murine class II Eα^k and Eβ^k	I-E^k	K^k, D^k	None	–
3T3 (murine fibroblast)	Murine class II Eα^k and Eβ^k	I-E^k	K^k, D^k	Cytochrome c	+

Class II MHC expression is required for antigen presentation to CD4+ antigen-specific T cells. In this experiment, a murine fibroblast cell line, 3T3, derived from an H-2^k mouse, which expresses class I, but not class II, MHC molecules, does not present cytochrome c to a cytochrome c–specific, I-E^k–restricted, T cell hybridoma line. When functional genes encoding the α and β chains of the I-E^k molecule are transfected into 3T3 cells, they become competent at presenting antigen to the T cell line.

Abbreviations: MHC, major histocompatibility complex; APC, antigen-presenting cell.